TABLE 4

Adenovirus recovery and purity in Process of the invention (Process 1) and Reference process

Process variant	Recovery TVP %	Recovery IVP %	HCP ng/ml	Total protein µg/dose	gDNA ng/dose	Total DNA ng/dose
Reference run 1	31/38	36	17	11/13	<LOD	6/8
Reference run 2	35/64	53	27	38/20	3	13/10
Reference average	42	45	22	20	<LOD - 3	9
Process 1 run 1	46/68	39	<LOD	13/11	<LOD	4/12
Process 1 run 2*	17	40	<LOD	10	<LOD	20
Process 1 average	37	40	<LOD	11	<LOD	14

*Analysis only performed once.

Two numbers indicate that the same sample was analyzed twice.

LOD = 1 ng/ml

[0084] A CaptoQImpres shallow salt gradient from 480 mM to 570 mM NaCl was critical to separate DNA fragments from virus particles. This corresponds to a 19% increase in salt concentration over the gradient or 7.5% increase in salt concentration per CV in the gradient (gradient calculation: 90 mM change over 2.5 CV, 36 mM/CV). The polishing with Capto core 700 as a second step resulted in a final bulk with a significant reduction in debris or impurities by Transmission electron microscopy (TEM) imaging compared to a reference process (Sepharose Q XL step elution followed by size exclusion, FIGS. **3**A and B, Table 4). The procedure is expected to perform equally well with a gradient of KCl or LiCl, or any combination of NaCl, KCl and LiCl.

[0085] From the above it clearly appears that Process 1 using a combination of Capto Q ImpRes anion exchange resin and Capto Core 700 resin has several advantages over the Reference process.

[0086] In smaller scale, Process 1 showed a clear advantage with regard to impurity reduction. In the scale-up experiments, Process 1 showed better HCP reduction (<LOD vs 22 ng/ml). This and other features makes the method of the invention a suitable method for purification adenoviral vectors for cell therapy.

[0087] Another major benefit of the invention was that up to 30 column volumes (CV) could be loaded the Capto Core 700 column in Process 1 whereas only 0.2 CV could be loaded in Reference process (150-fold higher load capacity). Furthermore, the yield was clearly better for polishing in Process 1 using the shell bead step compared to size exclusion chromatography, SEC (Table 3).

1. A method for adenovirus purification comprising the following steps: a) capturing adenovirus from an adenovirus-containing cell culture harvest on an anion exchanger resin; b) eluting said adenovirus with a shallow conductivity gradient with an increasing salt concentration of 15-25%, preferably 18-20%, over the gradient; c) adding said eluted adenovirus to a shell bead resin comprising a porous shell and a porous core, wherein the core is provided with hydrophobic interaction ligands and the shell is not provided with any ligands; and d) eluting said adenovirus from said shell bead resin in the flow through, wherein the adenovirus eluted in step d) comprises less than 1 ng/ml host cell protein (HCP).

2. The method according to claim **1**, wherein the salt is selected from NaCl, KCl and LiCl, or any combinations thereof.

3. The method according to claim **1**, wherein the salt is NaCl and the gradient is increasing 18-20% and the salt concentration is between 0-700 mM.

4. The method according to claim **1**, wherein the anion exchange resin is packed in a column and the shell bead resin is packed in another column, and wherein the adenovirus eluted from the anion exchanger resin is added to the shell bead resin in a volume corresponding to 15-30 column volumes (CV) of the column comprising shell bead resin.

5. The method according to claim **4**, wherein the adenovirus eluted from the anion exchanger resin is added to the shell bead resin in a volume corresponding to 25-30 column volumes (CV) of the column comprising shell bead resin.

6. The method according to claim **1**, wherein the porosity of the core and shell is the same of the shell bead resin.

7. The method according to claim **1**, wherein the porosity of the core and shell is different of the shell bead resin.

8. A composition comprising an adenovirus purified according to claim **1**, wherein the host cell protein (HCP) is below 1 ng/ml.

9. The composition according to claim **8**, wherein the adenovirus is an adenoviral vector suitable for cell therapy.

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